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CYTOLOGICAL TESTS OF INDEPENDENT ASSORTMENT AMONG HETEROMORPHIC CHROMOSOME PAIRS IN *DROSOPHILA MELANOGASTER**

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Mendel's second law, the law of independent assortment, states that when a number of pairs of hereditary factors are present, the pairs assort independently of each other. At the microscopic cellular level, the chromosomes are the bearers of these factors. Since this is the case, it should be readily possible to demonstrate that if pairs of contrasting alleles assort independently of each other, likewise so should the paired chromosomes on which these alleles are located. Yet evidence in support of this law, through cytological studies of the behavior of the chromosomes from parents to offspring in paralleling Mendel's factors, is far from being either numerous or conclusive.

Carothers (1913) described the relationship between the behavior of the X chromosome and that of the dyads of an unequal tetrad which occurred in the primary spermatocytes of the male orthopteran *Brachystola magna*. With the X chromosome of the male as a basis of comparison, she selected three hundred cells at random and found that the respective dyads assorted with the X chromosome in approximately a 1:1 ratio. In 1917, working with two genera of Acrididae (Orthoptera), Carothers described a number of instances in which the two homologous chromosomes differed morphologically from one another and as a pair were also distinguishable from the other chromosome pairs. The homologues of several pairs were traced through anaphase I and found to segregate at random in regard to both the X chromosome and each other, and presumably to the other eight pairs of chromosomes, thus demonstrating the Mendelian expectancy of random assortment. A few years later she made a number of matings whereby she could make a direct comparative analysis between the chromosomal constitution of the parents and that of the offspring, with respect to three heteromorphic pairs (Carothers, 1921). Due to the limited number of offspring obtained, however, no valid conclusions could be drawn on a statistical basis as to the manner in which the chromosome pairs were assorting.

King (1923) described the behavior of six heteromorphic chromosome pairs through anaphase I in 224 primary spermatocyte cells of *Pseudotrimerotropis thalassica*. Tracing the assortment of the six pairs in relation to the X chromosome and to each other, King found that the dyads and the X chromosome were assorting completely at random.

Thus, although the random assortment of homologous chromosomes has been well verified as to their segregation in both primary and secondary spermatocytes, documentation of the assortment of homologous chromosomes from parent to offspring has never been effected to the extent required for statistical analysis.

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The purpose of the present study was (1) to investigate cytologically the distribution of heteromorphic chromosome pairs from parent to offspring, first considering two pairs and then three pairs of chromosomes simultaneously, and (2) to determine whether the distribution among these pairs was completely random, thus satisfying Mendel's second law at the chromosomal level.

TABLE 1

Presence vs. absence of inversion loops in the salivary gland chromosomes when considering two heteromorphic chromosome pairs simultaneously

Inversion loop	X and II*	X and III*	II and III
Absent in			
Both	47	52	62
Only one	38	49	67
Only other	45	36	44
Neither	34	40	53
Total	164	177	226
Chi-square	2.6828	3.8135	5.4690
Probability	0.50–0.30	0.30–0.20	0.20–0.10

*Females only.

MATERIALS AND METHODS

The three stocks of *Drosophila melanogaster* selected for this study are symbolized by j92, g20, and h50. Each stock is homozygous for an inversion not present in the other two. (These stocks are listed in the Bloomington, Indiana stock list [Dros. Info Serv. 30, 1956], where stock g20 is listed as containing cn^2 and InCyR; however, these were not present in the stock received here.)

Stock j92 has a homozygous inversion in the X chromosome designated as In49. The inversion breaks occur between $4D_7$ and $4E_1$ and between $11F_2$ and $11F_4$. Phenotypically, stock j92 can be distinguished by the markers yellow (y) and vermilion (v) on the X chromosome and brown (bw) on the second chromosome.

Stock g20 has a homozygous inversion in the second chromosome designated as InCyL. The inversion breaks occur between $22D_1$ and $22D_2$ and between 33F and $34A_1$. Both markers of g20, black (b) and purple (pr), are located on this chromosome.

Stock h50 has a homozygous inversion in the third chromosome designated as sepInLR. This is a pericentric inversion which phenotypically "separates" the wings. As this inversion was not listed by Bridges and Brehme (1944), the exact location of the inversion was not known. However, from a study of the banding pattern, the inversion breaks occur at approximately 74C and 90E. This stock has two markers, pink peach (p^p) and radius incompletus (ri), both located on the third chromosome. The viability of these flies is considerably lower than that of the other two stocks and a great deal of care is needed to maintain the stock.

To obtain two heteromorphic chromosome pairs in each of three different stocks, protocols (1), (2), and (3) were used. Protocol (4) was used to obtain a stock heteromorphic for three chromosome pairs. In the latter protocol only those genotypes are listed which were involved in the subsequent crossings.

(1) Protocol involving stocks j92 and g20, resulting in the X and second chromosome pairs being heteromorphic:

P_1 y In49 v / y In49 v ♀♀ × bw / bw — + / b InCyL pr / b InCyL pr ♂♂

F_1 y In49 v / + ♀♀ × bw / b InCyL pr — y In49 v / bw / b InCyL pr ♂♂

F_2 female larval offspring to be classified.

(2) Protocol involving stocks j92 and h50, resulting in the X and third chromosome pairs being heteromorphic:

P_1 y In49 v / y In49 v ♀♀ × + / + — + / sepInLR ri p^p / sepInLR ri p^p ♂♂

F_1 y In49 v / + ♀♀ × sepInLR ri p^p / + — y In49 v / + ♂♂ × sepInLR ri p^p / + ♂♂

F_2 female larval offspring to be classified.

(3) Protocol involving stocks g20 and h50, resulting in the second and third chromosome pairs being heteromorphic:

P_1 b InCyL pr / b InCyL pr ♀♀ × + / + — + / sepInLR ri p^p / sepInLR ri p^p ♂♂

F_1 b InCyL pr / + ♀♀ × sepInLR ri p^p / + — b InCyL pr / + ♂♂ × sepInLR ri p^p / + ♂♂

F_2 male and female larval offspring to be classified.

(4) Protocol involving stocks j92, g20, and h50, resulting in the X, second, and third chromosome pairs being heteromorphic:

P_1 y In49 v / y In49 v ♀♀ × bw / bw — + / b InCyL pr / b InCyL pr ♂♂

F_1 y In49 v / + ♂♂ × bw / b InCyL pr — + / b InCyL pr / b InCyL pr ♀♀

F_2 y In49 v / + ♂♂ × bw / b InCyL pr — y In49 v / + / b InCyL pr / b InCyL pr ♀♀

F_3 y In49 v / y In49 v b InCyL pr / b InCyL pr + ♀♀ × + / + — + / + sepInLR ri p^p / sepInLR ri p^p ♂♂

F_4 y In49 v / + b InCyL pr / + sepInLR ri p^p / + ♀♀ × y In49 v / + b InCyL pr / + — sepInLR ri p^p / + ♂♂

F_5 female larval offspring to be classified.

The flies of each preliminary cross were raised at 24°C, whereas those of the final crosses, i.e., those crosses producing the larvae which were to be classified, were raised at 18°C. In these latter crosses, the parents were transferred every two or three days in order to avoid crowded culture bottles.

Each larva was first placed in a drop of saline solution on a slide and classified as to sex. The glands were dissected in aceto-orcein and were subsequently transferred to another slide containing a drop of aceto-orcein with Janus Green B (Cordeiro, 1957) and covered with a coverslide. The chromosomes were spread using a roller.

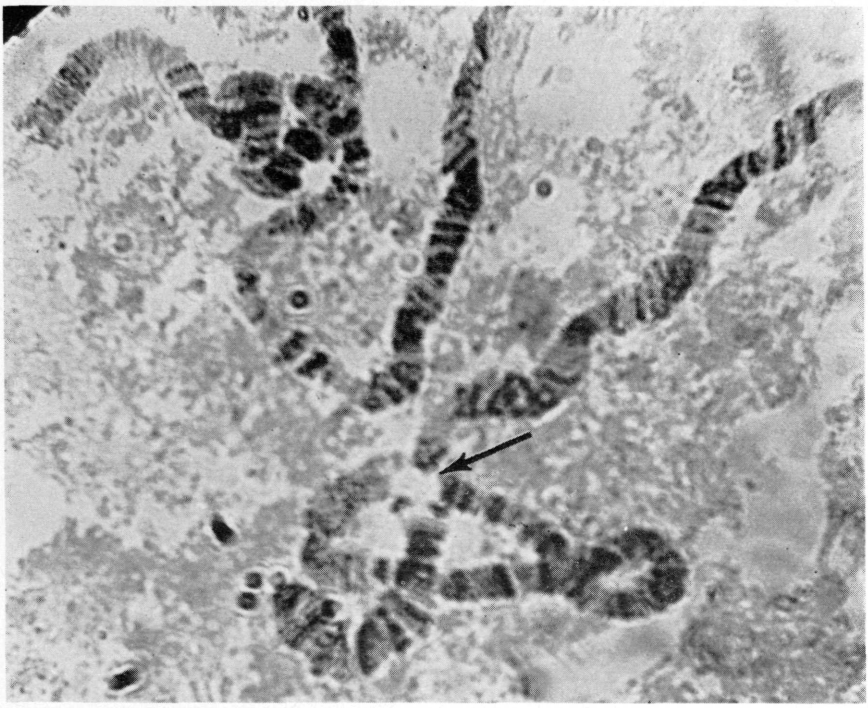

FIGURE 1. Photomicrograph of cell in which the X chromosome is heterozygous for the inversion designated as In49. Arrow indicates point of synaptic partner exchange. ×1440.

In a cursory examination, each preparation was scanned to record whether three, two, one, or no inversion loops were present. During a second and more thorough study of the slides, the chromosomes and their corresponding inversions, if present, were identified.

RESULTS AND DISCUSSION

The choice of *Drosophila melanogaster* for this study was prompted mainly by four considerations: (1) each chromosome pair is fully synapsed in the somatic salivary gland cells, and thus homologous chromosomes of one pair cannot be mistaken for those of another; (2) the banding of the chromosomes is constant and thus an aberration, if present, will always occur within the same known segment of bands; (3) since it is one of the most widely studied organisms in genetics, mutants with specific types of aberrations present are easily obtainable for experimentation and study; and (4) it is an ideal organism for genetics in that the life cycle is very short, the matings are easily controlled, offspring are produced in great numbers, even in pair matings, and there are only four pairs of chromosomes present.

Although all three inversions were quite large, I did not feel sufficiently versed in the reading of the chromosome band pattern to attempt the detection of homozygous inversions in the synapsed chromosomes. Instead, the chromosome pairs were grouped on the basis of whether inversion loops were present or absent. Thus, the larvae were grouped into four classes when two pairs of chromosomes were being studied, and into eight classes when all three pairs were involved.

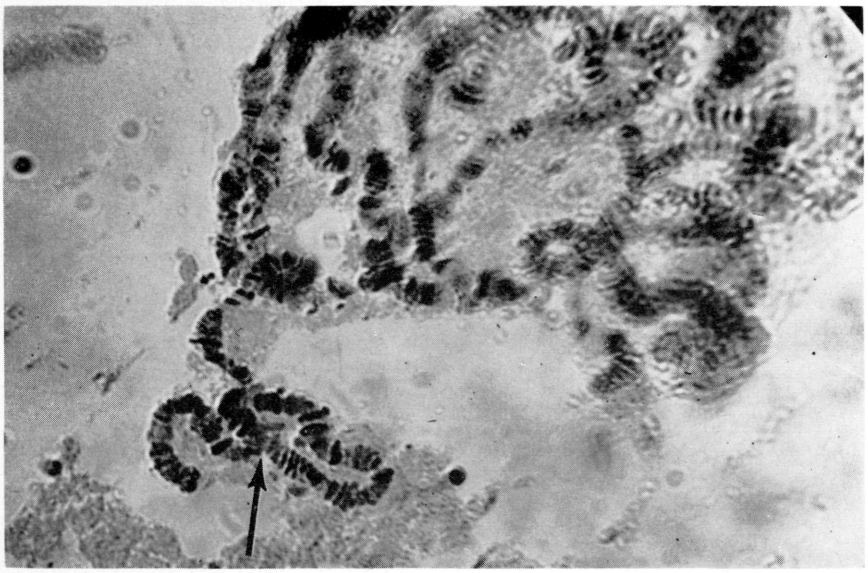

FIGURE 2. Photomicrograph of heterozygous inversion in the left arm of chromosome II, designated as InCyL. Arrow indicates point of synaptic partner exchange. ×1020.

With two pairs of chromosomes assorting independently the four classes of larvae should theoretically fall into a 1:1:1:1 ratio. With three pairs of chromosomes involved, the ratio becomes 1:1:1:1:1:1:1:1.

In the crosses respectively involving heteromorphic chromosome pairs X and II, X and III, and X, II, and III, only the females were classified as to the distribution of the chromosomes since in males the XY mechanism would permit but one class to appear with respect to the first pair of chromosomes.

Of the four final crosses, that between the F_1's from j92 crossed with g20 produced the best results from the statistical viewpoint of probability (see column

TABLE 2

Presence vs. absence of inversion loops in the salivary gland chromosomes when considering three heteromorphic chromosome pairs simultaneously

Inversion loop	X, II, and III*
Absent in X	
Absent in	
Both II and III	38
Only one	35
Only other	26
Neither	23
Present in X	
Absent in	
Both II and III	36
Only one	34
Only other	29
Neither	22
Total	243
Chi-square	8.8847
Probability	0.30–0.20

*Females only.

TABLE 3

Classification of offspring as to sex in a random sample from each of the four final crosses

Sex	X and II	X and III	II and III	X, II, and III
Male	182	199	105	215
Female	164	177	121	243
Total	346	376	226	458
Chi-square	0.9364	1.2872	1.1328	1.7118
Probability	0.50–0.30	0.30–0.20	0.30–0.20	0.20–0.10

TABLE 4

Segregation of the two homologues of heteromorphic chromosome pair III with respect to each other and to the other chromosome pairs

Inversion loop	With X	With II	With X and II	Total
Present	76	97	100	273
Absent	101	129	143	373
Total	177	226	243	646
Chi-square	3.5310	4.5310	7.6090	15.4800
Probability	0.10–0.05	0.05–0.02	<0.01	<0.01

Heterogeneity chi-square: 0.1955, probability 0.70–0.50.

TABLE 5

Segregation of the two homologues of heteromorphic chromosome pair II with respect to each other and to chromosome pairs X and III

Inversion loop	With X	With III	With X and III	Total
Present	79	106	114	299
Absent	85	120	129	334
Total	164	226	243	633
Chi-square	0.2196	0.8672	0.9260	1.9352
Probability	0.70–0.50	0.50–0.30	0.50–0.30	0.30–0.05

Heterogeneity chi-square: 0.0778, probability 0.80–0.70.

for X and II, table 1). This was probably due to the fact that both inversions are quite large and the corresponding inversion loops are readily seen even under low power. Therefore, few problems of interpretation arose with respect to this cross.

The results of the F_1 crosses between j92 and h50, and g20 and h50 are very similar (column for X and III and column for II and III, table 1). In both instances, the frequencies of the two classes where the inversion loop is present in chromosome III are lower than the other two classes. This is probably due to the fact that the inversion in chromosome III is pericentric and for that reason is somewhat hard to identify since the chromocenter usually is surrounded to some extent by the mass of chromosomes. Very infrequently, the chromosomes have been forced by the pressure of smearing to the side of the chromocenter and only in such an instance is the inversion, if not also forced over, clearly seen.

The results of the final cross involving three heteromorphic chromosome pairs simultaneously (table 2) have a fairly random distribution, except that again, classes which involve the presence of an inversion loop in chromosome pair III have consistently lower frequencies than the other classes.

Since the possibility exists that the presence of the inversions in themselves may have caused the segregation to deviate toward one class or the other, it was

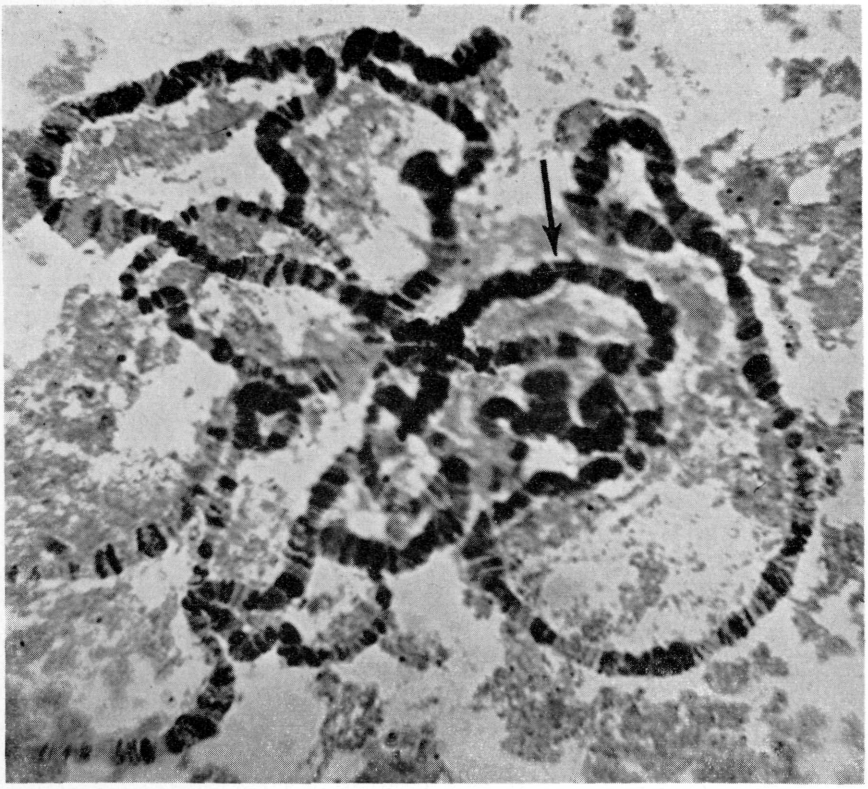

FIGURE 3. Photomicrograph of heterozygous inversion in chromosome III. This pericentric inversion, indicated by arrow point, is designated as sepInLR. ×1060.

considered necessary to ascertain if such were the case. As there is in *Drosophila* a "built-in" mechanism by which one can determine whether normal segregation is taking place, i.e., the XY mechanism, a random count was made, classifying male and female offspring in each of the final crosses. The results of this simple test are given in table 3. It may be seen that on the basis of a 1:1 expectation there is no significant deviation in any of these crosses.

Since the frequencies of the classes where the inversion loop is present in chromosome pair III are consistently low, it was thought necessary to find out whether the two homologues of this chromosome pair were segregating in a 1:1 ratio with respect to each other. In two of the three crosses, it was found that there is a significant deviation from expectation, in the direction of too few with a loop present, indicating that a 1:1 segregation of the two homologues was not taking place (see table 4). A chi-square heterogeneity test was also made, the results of which indicate that all three crosses are homogeneous with respect to the deviating segregation of chromosome pair III.

One reason mentioned above for this departure from the expected ratio may be the difficulty in detecting pericentric loops. An alternate explanation is that the marker genes and/or the inversion itself are somehow affecting the gametes. According to Bridges and Brehme (1944), the marker genes on chromosome III, i.e., p^p and ri, do not have any reduction of viability attributed to them. Therefore, assuming that the inversion is reducing the viability of those gametes which contain it, it can be shown that the inversion classes without loops will always exceed the inversion classes with loops by a factor of $4x^2/n^2$, where x/n is the

proportion of "lowering of gamete effectiveness" due to the presence of an inversion.

The values of x/n computed from table 4 are 1.4 percent, 1.2 percent, and 1.3 percent, for columns 1, 2, and 3, respectively. Therefore, the viability of those gametes which receive the inversion in chromosome III may be reduced by approximately 1.3 percent.

A similar series of chi-square tests (table 5) was made in regard to the two homologues of chromosome pair II. In these tests, there were no significant deviations from the expected ratio, and it may be assumed that these homologues are segregating at random with respect to each other. A chi-square heterogeneity test was also made, the results of which indicate that the three crosses are consistent with each other.

Thus, although the homologues of chromosome pair III were not segregating as expected, the deviation caused by this behavior was not great enough in any of the four crosses to affect the over all picture of independence in assortment.

SUMMARY

1. A cytological study of the salivary gland chromosomes of 810 larvae of *Drosophila melanogaster* was made in respect to the distribution of members of heteromorphic chromosome pairs from parents to offspring, as a test of Mendel's second law at the chromosomal level.

2. Inversions in the X, second, and third chromosomes were used as cytological markers.

3. The location of the inversion breaks of the sepInLR inversion in the third chromosome, previously unknown, are at approximately 74C and 90E.

4. Three F_1 stocks were developed, each with two heterozygously inverted chromosome pairs. The F_2 larvae of these stocks were studied. The results on the basis of chi-square tests showed no significant deviation from expectation based on the assumption that independent assortment was taking place.

5. A fourth stock was developed which was composed of flies heterozygous for all three inversions. The larvae from this heterozygous stock were also classified. As with the other three crosses, the results did not deviate significantly from those expected, assuming Mendel's second law was operating.

6. The two homologues of the third chromosome pair did not segregate in a 1:1 ratio with respect to each other, thus differing from the behavior of the X and second chromosome pairs. A possible explanation for this deviation is that the eggs and/or sperm which receive this inversion are not as viable as those which receive the noninverted third chromosome, i.e., a position effect.

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